Epidemiologic Considerations to Assess Altered DNA Methylation from Environmental Exposures in Cancer

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ABSTRACT: Epidemiologic studies in human populations have identified a broad spectrum of risk factors for cancer. Gene-damaging agents have been a primary focus of cancer epidemiology; however, all xenobiotics do not interact with DNA directly. Some exogenous agents induce epigenetic changes. In view of this, markers that measure changes to the epigenome must also be incorporated into molecular epidemiologic studies. We review the current understanding of the impact of exogenous agents including: micronutrients, chemotherapeutic agents, metals, and others, on DNA methylation. Two categories of genes are described: (1) genes that can alter susceptibility to aberrant DNA methylation and (2) genes that increase susceptibility to cancer when they are silenced through DNA methylation. Methods for incorporating markers of DNA methylation status into etiologic investigations of the impact of environmental exposures on disease (e.g., cancer) are discussed.

KEYWORDS: epidemiology; DNA methylation; epigenetics; environmental carcinogenesis

INTRODUCTION

There is growing evidence that DNA methylation and chromosomal histone acetylation are important determinants of gene transcription and that disorder in these expression control mechanisms is an important determinant in human carcinogenesis. Cells with abnormal DNA methylation acquire an overall gene expression pattern that favors proliferation and dedifferentiation, leading to neoplastic transformation.[1] Epigenetic variations operate through alterations in gene activation and expression, changes in chromosomal stability, and altered genomic imprinting,[2] resulting in modification of cell signaling pathways and cell growth.

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Although direct genetic damage to DNA in the form of mutations and structural chromosomal alterations has been the major focus of environmental carcinogenesis, exogenous exposures and inherited genetic susceptibility factors may also play a role in cancer induction via epigenetic pathways. Accordingly, we review evidence supporting the contribution of exogenous exposures, as well as inherited genetic susceptibility factors, to epigenetic mechanisms of carcinogenesis. The focus of our review is on environmental factors as determinants of DNA methylation status, as little is known about environmental exposures and histone acetylation. First, we briefly describe DNA methylation and its role in cancer.

DNA Methylation

Methylation of cytosine is the only naturally occurring modification of DNA in mammals. This genomic methylation occurs at the 5′ carbon of cytosine, mediated by one of three methyltransferases (*DNMT1, DNMT3A, DNMT3B*), with S-adenosyl methionine (SAM) as the methyl donor. Once methylated, 5-methyl cytosine (5-MeC) is maintained with a high degree of fidelity primarily by DNMT1 maintenance methylation. Only pharmacologic interventions with demethylating agents, such as 5-azacytidine, are capable of substantially altering cytosine methylation status.

In most cases, cytosine methylation occurs within CpG dinucleotides (a few non-CpG sequences also exhibit low-frequency methylation).[3] CpG sequences occur approximately once per 80 dinucleotides in 98% of the genome; however, about 2% of the genome is comprised of regions 200 bp to several kb in length, in which CpG dinucleotides occur at five times the frequency found in the genome as a whole (i.e., CpG islands). CpG islands are almost always located within gene promotor regions and exons.[3]

In young, healthy mammals, non-island CpG cytosine is almost universally methylated, whereas island CpG cytosine, with a few important exceptions, is almost universally unmethylated. The role played by high-frequency non-island CpG cytosine methylation is not understood, although it may enhance chromosomal stability and limit transposon activity, possibly associated with protection from infectious agents.[4] Island CpG cytosine methylation functions in gene silencing, as found in the promotor region of genes on the inactivated X chromosome (in females) and on inactivated imprinted genes (from either paternal or maternal origin). The mechanism of gene silencing through island CpG cytosine methylation in gene promotor regions is not fully established; however, steric hinderance of transcriptional machinery, recruitment of methyl-CpG–binding proteins that facilitate transcriptional repression (such as MeCP2, MBD1, MBD2, MBD3), and interaction with histone deactylases resulting in chromatin remodeling all appear to be involved.[5]

DNA Methylation in Cancer

In cancer, CpG island cytosine hypermethylation has been observed in more than 60 genes, including known tumor suppressor genes, implicating methylation-associated gene transcriptional silencing in carcinogenesis. The factors underlying CpG island hypermethylation are not understood; however, recent evidence suggests the existence of a CpG island methylator phenotype (CIMP) involving the silencing and inactivating of multiple genes by promoter hypermethylation,[6] possibly through

upregulation of *DNMT1*.[7] *DNMT1* is a maintenance methyltransferase (Mtase) and exhibits its effects on hemimethylated DNA; it is therefore surprising that *DNMT3b* (*de novo* Mtase) is not associated with CIMP. Colorectal carcinogenesis is frequently characterized by CIMP positivity. Even premalignant adenomas[8] and serrated adenomas[9] exhibit CIMP positivity, suggesting that this phenotype is an early event in colon carcinogenesis.[10] DNMT1 activity is generally elevated in transformed cells, offering a possible explanation for the altered methylation phenotype in tumorigenesis. Increased DNMT1 activity can occur before the appearance of the fully transformed phenotype, and it therefore has the potential to serve as an early disease marker.[1]

Hypermethylation at CpG sites can also predispose to mutation because 5-MeC can spontaneously undergo hydrolytic deamination, causing C-to-T transitions. This type of enhanced mutagenesis is seen in the germline of all organisms that methylate DNA. Increased mutation rates, such as those observed at CpG sites in the p53 gene,[11] have been associated with endogenous and exogenous exposures to mutagens. The transfer of methyl groups from S-adenosylmethionine (SAM, the universal direct methyl group donor) to many different methyl group acceptors, is catalyzed by enzymes known as methyltransferases. Under *in vitro* conditions in which the concentration of SAM is limiting, enzymatic deamination of cytosine to uracil has been observed.[12]

Hypomethylation of cytosines is also a predominant feature of many cancer types. However, it occurs primarily in repetitive sequences that have no obvious impact on gene expression. Hypomethylation of nonpromoter regions of DNA and of structural elements such as centromeric DNA may potentially lead to genetic instability.[13]

Environmental Exposures

Exogenous agents, including cigarette smoke, dietary factors, occupational and environmental chemical exposures, and biologic agents, are causative factors in many cancers. The scientific focus of molecular epidemiology is the elucidation in human populations of the biologic pathway linking exposures to disease, with consideration of internal dose and early biologic effects, mediated by susceptibility factors and assessed by progressive somatic damage at the organ site. Genetic and chromosomal damage and protein alterations are established markers of these early biologic effects and of cancer. With increasing data on epigenetic changes associated with environmental exposures, epigenetic damage also needs to be included in the molecular epidemiologic model, as illustrated in FIGURE 1.[14] Here, we review the current state of understanding of environmental epigenetics.

Micronutrients

Epidemiologic studies suggest that low dietary folate, possibly in combination with increased alcohol intake, increases the risk of a number of different cancers.[15,16] Folate is essential for *de novo* biosynthesis of purines and thymidylate. Folate acts as an important mediator of methyl group transfer, which is necessary both to maintain DNA integrity and for the synthesis of SAM, the universal methyl donor.[17] Folate deficiency could contribute to cancer risk by increasing the rate of

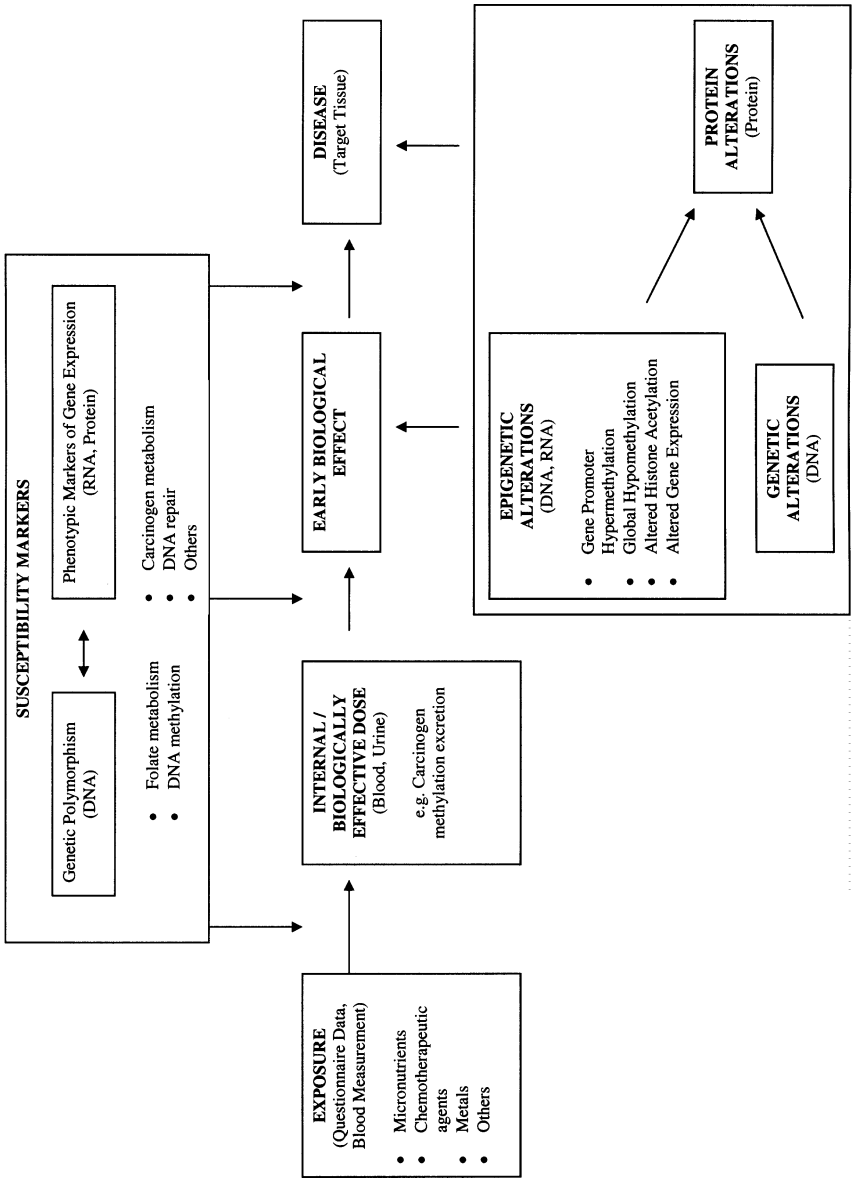

FIGURE 1. Schemata incorporating epigenetic markers into the molecular epidemiologic model (adapted from the Committee on Biological Markers of the National Research Council[117]).

chromosomal breakage caused by uracil misincorporation during DNA synthesis or by decreasing DNA methylation.[18] Exposure to the drug 5-deoxyazacytidine, an established inhibitor of methyltransterase, and dietary deficiency of choline, methionine, and folate can demethylate DNA and cause increased chromosome breaks.[19] Vitamins B_6 and B_{12} are also important cofactors required in the folate 1-carbon metabolism pathway.

Dietary folate deficiency results in elevated levels of plasma homocysteine, increased DNA hypomethylation, and increased dUTP/dTTP ratios in mitogen-stimulated lymphocyte DNA, suggesting uracil misincorporation into DNA.[20] Animal studies have shown that folate deficiency increases the risk of colorectal neoplasia, presumably because of its effect on DNA methylation.[21–25] Folate deficiency causes chromosomal damage in lymphocytes *in vitro*[26] as well as in lymphocytes and buccal cells in female volunteers.[27] It has been suggested that insufficient dietary intake of folate and B_6 could contribute to the increased cancer risk observed in that portion of the population that eats the fewest fruits and vegetables.[28,29] Dietary deficiency of micronutrients that are not found primarily in fruits and vegetables, including B_{12}, may also account for elevated cancer risks in portions of the population.

Chemotherapeutic Agents

Some of the earliest work in exogenous exposure and DNA methylation was conducted in the study of chemotherapeutic agents. Genetic instability in tumor cells is thought to play a major role in tumor progression, metastasis, and the development of resistance to anticancer agents.[30–32] In addition, epigenetic changes in tumor cells have been shown to exert comparable effects on cancer progression. Thus, researchers have demonstrated that a wide variety of commonly used cancer chemotherapy agents induce profound changes in DNA methylation patterns in several human tumor cell types *in vitro*. Drug-induced DNA hypermethylation silences gene expression during the period of drug-induced toxicity. Furthermore, such induced hypermethylation can lead to drug resistance by randomly inactivating genes whose products are required to activate cancer chemotherapy agents to their cancer-killing forms.

These chemotherapy-induced epigenetic changes have been observed both *in vitro* and *in vivo*.[30] Of the agents tested, cisplatin, a DNA cross-linking agent, was the most potent inducer of DNA hypermethylation, possibly owing to the ability of cisplatin adducts to induce conformational changes that render DNA a better substrate for DNA cytosine 5-methyltransferase. DNA hypermethylation could also be induced by exposure to the following agents: the antibiotic doxorubicin; aneuploidy-inducing microtubule inhibitors such as vicristine, vinblastine, and colchicine; and antimetabolites such as methotrexate.[30] Researchers also found that chemotherapeutic drug-induced DNA hypermethylation could be blocked, in a dose-dependent manner, by preexposure to hypomethylating agents such as 5-aza-2′-deoxycytidine. In contrast, topoisomerase II inhibitors such as nalidixic acid, novobiocin, etoposide, and teniposide inhibited DNA methylation, possibly by inducing conformational changes that convert the affected DNA into a poorer substrate for this enzyme.[32] In addition, alkylating agent exposure appeared to modify DNA, resulting in DNA hypomethylation, possibly by inactivating the sulfhydryl rich DNA methylase enzyme.[33]

Metals

Nickel

Although the main hypothesis for metal-induced carcinogenicity is that it occurs via inhibition of DNA repair, exposure to several metals and metalloid elements also alters DNA methylation and gene expression.[34,35] Studies of nickel carcinogenicity have been conducted for several decades in both cell and animal models.[36] In experimental animals, nickel compounds can be very potent carcinogens, but nickel itself is not considered mutagenic.[37] On the other hand, nickel induces a high incidence of cancer at the site of administration. In CHO cells, crystalline nickel sulfide silences tumor suppressor and senescence genes by enhancing DNA hypermethylation on the X chromosome.[38] Nickel also induces gene methylation in signaling pathways and may be important for cancer cell survival. The importance of the chromosomal position of altered genes relative to heterochromatin has also been noted. For example, nickel compounds cause DNA hypermethylation in a transgene associated with heterochromatin (but not the same transgene distant from heterochromatin), thereby altering expression of transcription factors such as activating transcription factor–1 (ATF-1), RB (retinoblastoma), HIF-1 (hypoxia-inducible factor 1). The ability of nickel to induce DNA methylation is probably secondary to its binding to the phosphate backbone of DNA in place of Mg^{2+} within condensing DNA and chromatin. The additional condensation may trigger more *de novo* DNA methylation and inactivation of gene expression.[37] Nickel may also cause gene silencing by binding to histone H4, subsequently inhibiting lysine acetylation.[39] Such signaling pathways may be important for the survival of cancer cells.

Arsenic

Arsenic, a carcinogenic metalloid element found in environmental and occupational settings, induces hyper- and hypomethylation *in vitro*.[40,41] Epidemiologic studies show that inorganic arsenic is associated with increased risk of skin, bladder, lung, liver, and kidney cancers.[42] Arsenicals likely act without direct interaction with DNA, with the exception of dimethylarsenic (DMA) at very high doses.[43] Inorganic arsenic has been shown to induce gene amplification *in vitro*.[44] However, *in vivo* studies of exposed and unexposed bladder tumors did not demonstrate significant differences in the prevalence of gene amplification.[45] The toxicologic effects of arsenic are multifaceted:[48–53] arsenic inhibits DNA repair enzymes *in vitro*[46] and *in vivo*;[47] it alters normal cell division by disrupting tubulin proteins in the mitotic spindle; and it induces both kinetochore and centromere–positive and –negative micronuclei *in vitro* and *in vivo*. The *in vitro* effect of arsenic on DNA repair results from alterations in DNA methylation patterns.[54] Thus, arsenic-induced inhibition of DNA repair, with associated malignant transformation, is paralleled by DNA hypomethylation and altered gene expression.[40] In A549 type II lung epithelial cells cultured in the presence of arsenic, researchers discovered a dose-response hypermethylation pattern in a 341–base pair fragment of the p53 promoter.[41]

Arsenic is enzymatically methylated primarily in the liver, a reaction that requires SAM and Mtases. Like nickel, arsenic activates transcription factors such as AP-1 and induces oncogenes including c-fos, c-jun, and c-myc.[55–57] The relevance of these findings in relation to arsenic-exposed humans or experimental animals has yet to be

evaluated. However, increasing evidence supports the hypothesis that arsenic shares many properties of tumor-promoting chemicals by affecting specific cell signal transduction pathways involved in cell proliferation.[58–60] It is not known whether these changes occur through epigenetic mechanisms.

Also, arsenic, like other epigenetic carcinogens, appears to alter genetic stability. *In vivo*, bladder tumors from individuals previously exposed to high levels of arsenic showed more genetic changes and aneuploidy than tumors from unexposed individuals. This suggests that the tumors from arsenic-exposed patients exhibited a genetically more unstable phenotype than tumors from nonexposed cases even after controlling for tumor stage and grade.[45] Whether the genetic instability associated with increasing exposure occurs through epigenetic mechanisms has not been determined.

Methylation is also required for the detoxification of arsenic. The amounts of total and speciated arsenic in the urine can provide a quantitative measure of current exposure and can be used as a phenotypic marker of biotransformation and excretion of methylated metabolites. Arsenic in drinking water is generally ingested in the pentavalent inorganic form and is subsequently methylated, first to monomethylated arsenate and then to dimethylated arsenite. The methyl groups are derived from SAM and the folate 1-carbon metabolism pathway through the same processes as those involved in DNA methylation. Specific arsenic methyltransferases have not yet been identified, but some of the enzymes involved in arsenic methylation could belong to the glutathione S-transferase superfamily.[61] It has been reported that methylation can occur in the presence of B_{12} and glutathione alone.[62]

Variations in interindividual methylation capacity have been demonstrated in population studies in arsenic-endemic regions.[63–66] The thinking had been that methylation is a detoxification mechanism for arsenic because methylated metabolites are excreted faster, are less reactive with tissues, and are less cytotoxic.[67–69] However, recent studies suggest that methylation may increase rather than decrease arsenic's toxicity.[70] The methylation of inorganic arsenic produces short-lived trivalent forms of arsenic that appear to inhibit enzyme activity, increase cell toxicity, and have genotoxic properties that may contribute to cancer development.[71–73] Thus, there are three possible outcomes of methylation as a biotransformative process that may alter cancer susceptibility:

(1) methylation is efficient and protective, leading to detoxification and excretion of arsenic from the body, lowering tissue-specific dose and toxic effects of inorganic arsenic;
(2) methylation forms more toxic metabolites of arsenic that may be more reactive with tissues and promote cancer; or
(3) methylation of arsenic creates competition with DNA methylation by competing for methyl donation from SAM, resulting in DNA hypomethylation.[40,70]

Findings of a recent study argue that it is unlikely that the quantity of SAM required for methylation of arsenic is capable of reducing the SAM supply to an extent great enough to result in DNA hypomethylation.[74] However, two studies have found that individuals with arsenic-induced skin cancer (cases) have poorer arsenic methylating capacity than do controls.[75,76] In an experimental animal model, arsenic methylation was directly affected by nutritional status. It was found that arsenic

methylation could be decreased by altering methylation either through low intake of SAM precursors or through methylation inhibition.[68,77]

Selenium

Selenium, like arsenic, is detoxified through methylation pathways catalyzed by methyltransferases;[78] however, selenium is considered to have anticarcinogenic properties at subtoxic doses.[71,74,78–80] In cell culture models, pre- or coexposure to selenium enhances arsenic toxicity by increasing arsenic tissue retention and suppressing its methylation.[81] Both selenium and arsenic affect DNA methylation, suggesting that competition for methyl donation from SAM occurs among cytosine DNA methyltansferase, selenium, and arsenic.[74,82]

Cadmium and Chromate

Cadmium and chromate are similar to arsenic and nickel in that they do not cause direct DNA damage, but appear to cause DNA protein crosslinks, aberrant gene transcription, and genetic instability and to interfere with DNA repair.[83–85] Chromium exposure is associated with increased microsatellite instability[83] and increased p53 mutation prevalence[86] in tumors. Nevertheless, a specific epigenetic mechanism has not yet been demonstrated for these metals.

Other Exposures

Several other agents alter DNA methylation patterns *in vivo*. Disinfection byproducts resulting from the disinfection of drinking water, including chloroform, dichloroacetic acid, and trichloroacetic acid, show an ability to alter DNA methylation.[87] These chemicals are known mouse liver carcinogens, but it is not known whether they are also human carcinogens.

Tobacco smoke alters DNA methylation. Methylation of the promoter of the estrogen receptor (ER) gene has been reported in lung tumors from smokers of tobacco.[88] The same paper reported a study of A/J mice, which are sensitive to cancer induction by the tobacco-specific carcinogen 4-methylnitrosamino-1-3-pyridyl-1-butanone (NNK). Tumors in mice that had been exposed to NNK had a much lower incidence of ER methylation than tumors in unexposed mice. In contrast to the effect of NNK in these A/J mice, plutonium induced tumors with a higher incidence of ER methylation, and X-rays induced tumors that had an intermediate frequency of ER methylation.[88] In a recent study of human hepatocellular carcinoma, *p16* methylation was significantly different in tumors from China and Egypt compared to tumors from the United States and Europe.[6] Similar geographic variation was observed for ER methylation and CIMP. This study demonstrates that geography-based etiologic factors can influence the epigenetic configuration of a specific histologic tumor type, hepatocellular carcinoma.

SUSCEPTIBILITY POLYMORPHISMS AND MARKERS

Functional polymorphisms in genes involved in the human folic acid metabolic pathway may be involved in cancer, as several genes in this pathway are also impor-

tant in determining the availability of nucleotides for DNA synthesis and methylation. The variant form of the C-to-T polymorphism at codon 677 of the 5,10 methylene tetrahydrofolate reductase (MTHFR) gene was recently shown to be protective against colon and bladder cancer[15,89] and acute leukemia[90] in adults. Other polymorphisms have been demonstrated in genes in this pathway, including an A-to-G polymorphism at codon 2756 of the methionine synthase (MS) gene,[91–93] an A-to-G polymorphism at codon 66 in the methionine synthase reductase gene,[94] and a T-to-C polymorphism at codon 833 of the cystathionine beta-synthase (CBS) gene.[93,95] However, the actual relationship between these genes and DNA methylation has not been resolved. Bioavailability of methionine, a known precursor for SAM, is also under study.

Evidence does exist, however, for an interaction between enzyme polymorphisms that confer altered activity and exogenous toxic exposures in relation to disease outcomes. For example, insufficient enzyme activity was shown to increase the toxicity of an exogenous chemical in a case study of a 16-year-old girl with homocysteinuria. Although generally caused by deficient activity of cystathionine-B synthase, in this case homocysteinuria resulted from deficient MTHFR activity. All family members had a similar history of exposure to the pesticide copper acetate arsenite; yet, only the patient developed severe clinical signs and symptoms of arsenic poisoning. The MTHFR enzyme deficiency may have severely reduced her ability to methylate and biotransform arsenic through the normal detoxification pathways, while her family remained symptomless.[96]

In analogous fashion, polymorphisms in genes that regulate DNA methylation and histone acetylation are candidate markers for methylation-mediated cancer susceptibility. An example of such a candidate marker is the chief enzyme responsible for propagating DNA methylation patterns in adult vertebrate cells, DNA methyltransferase 1 (DNMT1).[97–99] This gene, while highly conserved, does exhibit differential splicing, resulting in multiple protein isoforms. Furthermore, mutation of DNMT1 results in embryonic lethality in mice.[100] Other candidate genes include those that code for proteins that complex with DNMT1, such as DNA methyltransferase 1–associated protein 1 (DMAP1) and histone deacetylase (HDAC) 2. Researchers are only beginning to understand the processes that cause variability in DNA methylation, histone acetylation, and gene expression.[13]

A recent study reported that subjects carrying functional polymorphic variants of glutathione S-transferase P1 or NADPH quinone oxidoreductase had an increased risk of aberrant promoter methylation of the O^6-methylguanine-DNA methyltransferase (MGMT) DNA repair gene and the $p16^{INK4a}$ tumor suppressor gene. These observations suggest that genetic variation may play an important role in determining an individual's ability to metabolize carcinogens related to methylation-mediated carcinogenesis.[101] In addition, susceptibility genes that are polymorphic in germline can be inactivated through methylation in specific somatic tissues. For example, methylation of the cytosines in the 5′-regulator region of *GSTP1* is associated with loss of expression of the GSTP1 protein. This phenotype is the most common epigenetic alteration found in prostate adenocarcinoma,[102–105] being detected even in the precursor lesion, prostatic intraepithelial neoplasia (PIN), but not in benign prostatic hypertrophy (BPH).[106]

Genetic polymorphisms in genes involved in pathways that modulate and repair DNA damage after carcinogen exposure may also determine the occurrence of *de*

novo promoter methylation. Among the enzymes belonging to the major pathways involved in removal and repair of methylation and oxidative DNA damage[107] are MGMT and base excision repair enzymes. MGMT, when inactivated through germline mutation or promoter methylation in somatic tissues, fails to remove the methyl group at the O^6 position of guanine, resulting in G:C-to-A:T transitions during DNA replication.[108,109] MGMT-knockout mutants also exhibit increased rates of mutation and cancer when exposed to methylating agents.[110]

Base excision repair enzymes participate in cellular protection against methylating agents, oxidative compounds, and ionizing radiation in a multistep process involving several repair proteins, including methylpurine DNA glycosylase (MPG), human 8-oxoguanine DNA glycosylase (hOGG1), and apurinic/apyrimidinic endonuclease 1 (APE1).[111,112] Recent evidence suggests an intimate correlation among mismatch repair genes, genetic instability, and methylation capacity in colon cancer cell models.[109,113] Methylation-mediated silencing of the mismatch DNA repair gene hMLH1 was found in sporadic cases of colorectal, endometrial, and gastric tumors. Such hMLH1 methylation may possibly precede expression of the CpG island methylator phenotype (CIMP),[113,114] although existing data do not fully support this sequence.

EPIDEMIOLOGIC APPROACHES TO UNDERSTANDING DNA METHYLATION AND CANCER

Epidemiologic studies have related cancer risk to environmental, biochemical, and genetic risk factors. With advances in understanding the molecular underpinnings of cancer, it becomes increasingly possible to evaluate risks in relation to specific molecular phenotypes of cancer. Archival paraffin-embedded and freshly frozen pathology samples are useful resources for evaluating environmental risk factors in relation to gene-specific and global methylation status in tumors. Developments in microarray technology will facilitate more informative use of these resources. With the growing ability to detect aberrant DNA methylation in serum, plasma, lymphocytes, and normal and cancerous tissue, methylation markers may serve as biomarkers of exposure, early epigenetic change, and disease susceptibility, allowing for the evaluation of environmental, biochemical, and genetic factors in relation to disease outcome.

In FIGURE 1, two sets of markers for disease susceptibility are presented. These include: (1) genetic markers of functional polymorphisms in genes involved in folate metabolism, DNA methylation, carcinogen metabolism, and DNA repair; and (2) phenotypic markers of altered gene expression. Many opportunities exist to examine relationships among these markers in cross-sectional studies of healthy individuals currently exposed to many of the agents previously described in this manuscript. For example, cross-sectional studies have been conducted to investigate the relationship between arsenic exposure and interindividual variations in methylation and detoxification pathways.[66] If promoter hypermethylation or global hypomethylation were outcomes of interest, as early markers of effect, surrogate media such as blood, exfoliated cells, or other noninvasively obtained tissue might be used to estimate epigenetic consequences of exposures. The development of such phenotypic markers is critical because diseased tissue is often not available in healthy individuals. Also in

such studies, markers of genetic susceptibility can be measured in genomic DNA from blood or buccal cells, and expression of susceptibility genes can be measured in blood or tissue. Dietary intake of important micronutrients involved in methylation pathways must also be considered.

In summary, epigenetic markers of disease can now be added to the previously established markers of early biological effect and disease. Epigenetic alterations of early biological effect and disease are recognized as some of the earliest detectable changes in individuals at very high risk for cancer.[101,103,115,116] Furthermore, gene methylation changes, a specific epigenetic alteration, can result in aberrant gene and protein expression. Therefore, quantification of RNA and proteins in target or surrogate tissues may be used as phenotypic markers of altered methylation status of genes that encode for them. Marker prevalence can be correlated with exposures to carcinogens of interest. Intervention studies could be conducted to determine if removal of exposure results in fewer epigenetic changes in exposed tissues.

As indicated by the research reviewed here, epigenetic mechanisms may occur at an early stage of carcinogenesis and may be related to an exposure-specific tumor phenotype (FIG. 1). Epidemiological studies provide opportunities to elucidate disease risks caused by exposure to epicarcinogens. Unlike classic carcinogens, epicarcinogens do not physically alter the nucleic acid sequence of DNA. Incorporating nutritional assessment, susceptibility, gene expression, and tissue analyses studies will contribute to our understanding of environmental/occupational exposures, the epigenome, and cancer.

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